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Fate of fumonisins in cattle fed contaminated feed

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FATE OF FUMONISINS IN CATTLE FED CONTAMINATED FEED

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Summary

Fumonisin are water-soluble carcinogenic mycotoxins produced by many species of *Fusarium* molds. Fumonisin occur widely in corn, making them a problem in corn-based feed. Their toxicity has been established in many species. However, their effects on cattle and the potential of carryover to the human diet through beef has not been studied extensively. A 30-day cattle feeding study was conducted by feeding fumonisin-contaminated corn grits dosed at 400 g/g fumonisin B₁ (FB₁) and 130 g/g fumonisin B₂ (FB₂) to 3 steers averaging 480 lb. Premortem analysis involved urinalysis; tests for liver functionality; and analysis of the blood, urine, and feces for the presence of fumonisins or their metabolites. Postmortem analysis involved necropsy, analysis of tissue for fumonisins, and histopathology. The test animals showed some slight liver abnormalities. The feces contained unmetabolized FB₁ and FB₂ ($\geq 80\%$ of the fed dose), and trace amounts were detected in the urine. Tissue analysis resulted in detection of 2.1 g/g FB₁ in the liver, 0.1 g/g FB₁ in the muscle, and 0.02 g/g FB₁ in the kidney, indicating a high feed:tissue ratio, and consequently insignificant carryover into the human diet.

(Key Words: Fumonisin, Toxicity, Residues in Tissues.)

Introduction

The toxicological effects of fumonisins have been established in horses, swine, rats, and nonhuman primates. However, their effect(s) on ruminants remain undetermined. The unique physiology of the ruminant and its microbes may alter the fumonisin toxicology normally seen in nonruminant species. The evidence for

such alteration in cattle is their tolerance to fumonisin exposure at levels fatal to horses and swine. The common occurrence of fumonisins in corn and the suspected tolerance of cattle to fumonisins potentially could result in chronic fumonisin exposure in cattle consuming corn-based diets, could lead to residues of fumonisins or their metabolites in cattle tissues, and could carry over to the human diet via consumption of fumonisin-contaminated beef. Our study was designed to test that hypothesis.

Experimental Procedures

Animal Feed and Dose

Six individually penned Holstein steers were acclimated for a period of 10 days before the start of the study and were weighed before, midway, and at the end of the feeding study. Three steers were used as test animals, and the remaining three served as controls. They were fed twice daily for 30 days on a diet consisting of a 85:15 (wt/wt) mix of dairy herd-mix and alfalfa hay. Daily consumption was 2% of body weight. The fumonisin-contaminated grits were prepared by inoculating previously sterilized corn grits with a high-fumonisin producing strain (*Fusarium moniliforme* M1293) and incubating them in a greenhouse for 25 days. Before feeding, the fumonisin-contaminated grits were mixed manually with the daily ration to obtain a daily dose of 400 g/g fumonisin B₁ (FB₁) and 130 g/g fumonisin B₂ (FB₂). On day 31, the animals were sacrificed, and necropsies were done to check for presence of gross lesions. The visceral organs, muscle, and fat were analyzed for the presence of fumonisins and their metabolites. Histopathology, involving tissue examination by both light and electron microscopy, was done on selected visceral organs.

Clinical Chemistry and Urinalysis

Premortem collection of blood, feces, and urine samples was done in the morning before the animals were fed on days 3, 0, 7, 14, 21, and 28 of the study. The steers were bled via jugular venipuncture, and the blood was analyzed for aspartate amino transferase (AST), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), bilirubin, and cholesterol to evaluate liver function. Urine was analyzed for creatinine, color, specific gravity, glucose, bilirubin, ketone, blood, pH, and protein and microscopically examined for erythrocytes, leukocytes, casts, epithelial cells, crystals, and bacteria.

Sample Preparation

Blood and urine. Blood (10 cc) was centrifuged for 15 min to separate the plasma layer. The plasma proteins were precipitated by addition of methanol and separated by centrifuging for 15 min. The resulting supernatant was used for solid-phase extraction (SPE) cleanup prior to analysis.

Feces, visceral organs, and meat. Ten g of sample (feces, liver, spleen, kidneys, gall bladder, gastrohepatic lymph nodes, pancreas, tongue, sub-cutaneous fat, kidney-fat, longissimus dorsi, chuck, and round) were blended at high speed with 75 ml of acetonitrile:water (1:1, v/v). The resulting supernatant was used for SPE cleanup.

Analysis of fumonisins. All chromatographic analyses were done using high performance liquid chromatography (HPLC), with a C-18 reversed phase column and fluorescence detection. Samples were analyzed for FB₁, FB₂, and their metabolites resulting from full or partial hydrolysis.

Results and Discussion

Each of the test animals was exposed to an average of 25,830 mg of FB₁ and 8,430 mg of FB₂ during the 30-day feeding period. The test animals showed no sign of feed refusal and consumed all of their feed before the next feeding period. Body temperatures taken on days -5, -4, -3, -2, -1, 0, 1, 2, 3, 4, 7, 14, 21, and 28 were unaffected by fumonisin-contaminated feed. No significant body weight differences occurred.

Evidence of liver stress can be construed from changes in the liver function tests, namely serum AST, GGT, LDH, and cholesterol. These changes suggest some hepatotoxicity at the molecular level, an effect common to all species affected by fumonisins. The feces showed the highest levels of FB₁ (127 g/g) and FB₂ (35 g/g). No evidence of any metabolized fumonisins was found in the feces. Traces of the intact fumonisins were detected in the urine.

No significant gross lesions were noted in any of the tissues of the test and control animals. The organ-to-body weight ratios for heart, brain, lungs, liver+ gall bladder, kidneys, spleen, and pancreas were normal. Fumonisin B₁ was found in muscle (0.1 g/g), liver (2.1 g/g), and kidneys (0.02 g/g). Fumonisin were not detected in the fat, tongue, gall bladder, spleen, pancreas, or the gastro-hepatic lymph node. Table 1 shows the amounts of FB₁ residue detected in the tissues and their corresponding feed:tissue ratios.

In conclusion, cattle exhibit a high feed:tissue ratio of fumonisin, and, therefore, can tolerate levels in feed that are normally fatal to horses or swine. The majority of the dose was excreted as the unmetabolized parent molecule in the feces. Fumonisin were not detected in the blood at 12 h or more after feeding, and only trace amounts were detected in the urine. Thus, carryover of FB₁ from cattle consuming fumonisin-contaminated feed to the human diet via consumption of beef does not seem to be a problem.

Table 1. Fumonisin Residues in Tissues from Cattle Fed Dietary FB₁ and FB₂

Steer No.	Levels of FB ₁ + FB ₂ in Feed ^a	Liver		Muscle ^b		Kidney ^c	
		FB ₁	F/T ^d	FB ₁	F/T	FB ₁	F/T
	(g)	(g/g)		(g/g)		(g/g)	
T-1	27.2 + 8.9	4.6	1,600	.52	1,702	.03	1.0 10 ⁶
T-2	24.5 + 8.0 ^b	0.11	81,666	.15	61,250	.01	2.8 10 ⁶
T-3	24.5 + 8.0	1.5	6,125	.64	2,227	.02	1.2 10 ⁶

^aAnimals were fed the fumonisin contaminated diet for 30 days.

^bCalculations based on 50% of total body weight being edible tissue.

^cCalculations based on weight of both kidneys.

^dFeed to tissue ratio (F/T) - overall level of fumonisins in feed divided by the level in the specified tissue.